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Serine/Threonine Protein Kinases from Bacteria, Archaea and Eukarya Share a Common Evolutionary Origin Deeply Rooted in the Tree of Life

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Abstract

The main family of serine/threonine/tyrosine protein kinases present in eukarya was defined and described by Hanks *et al.* in 1988 (*Science*, **241**, 42–52). It was initially believed that these kinases do not exist in bacteria, but extensive genome sequencing revealed their existence in many bacteria. For historical reasons, the term “eukaryotic-type kinases” propagated in the literature to describe bacterial members of this protein family. Here, we argue that this term should be abandoned as a misnomer, and we provide several lines of evidence to support this claim. Our comprehensive phylostratigraphic analysis suggests that Hanks-type kinases present in eukarya, bacteria and archaea all share a common evolutionary origin in the lineage leading to the last universal common ancestor (LUCA). We found no evidence to suggest substantial horizontal transfer of genes encoding Hanks-type kinases from eukarya to bacteria. Moreover, our systematic structural comparison suggests that bacterial Hanks-type kinases resemble their eukaryal counterparts very closely, while their structures appear to be dissimilar from other kinase families of bacterial origin. This indicates that a convergent evolution scenario, by which bacterial kinases could have evolved a kinase domain similar to that of eukaryal Hanks-type kinases, is not very likely. Overall, our results strongly support a monophyletic origin of all Hanks-type kinases, and we therefore propose that this term should be adopted as a universal name for this protein family.

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In a landmark paper from 1988, Hanks *et al.* [1] performed a multiple sequence alignment of known Ser/Thr/Tyr protein kinases catalytic domains. A common structural feature of these proteins is a 250- to 300-amino-acid catalytic domain, containing 11 conserved sequence motifs that constitute the active site. Initially, protein kinases of this type were identified only in eukarya. When *Escherichia coli* genome was sequenced in 1997 [2], no genes encoding the canonical Hanks-type kinases were found. It was previously known that *E. coli* possesses a different

type of Ser/Thr kinase, the bifunctional isocitrate dehydrogenase kinase/phosphatase [3]. However, genome sequencing of other bacteria, such as *Myxococcus xanthus*, *Mycobacterium tuberculosis*, *Bacillus subtilis*, *Helicobacter pylori* and *Haemophilus influenzae*, provided evidence for existence of Hanks-type kinases, which was subsequently confirmed by biochemical characterization [4]. Today, Hanks-type kinases are known to control many important processes in bacterial physiology via protein substrate phosphorylation [4]. To provide just a few

examples, in *M. tuberculosis*, kinases PknG and PknH contribute to virulence and survival during the infection process [5,6]. Similarly, in *Yersinia pseudotuberculosis*, a secreted kinase YpkA is required for virulence [7]. In *Streptococcus pneumoniae*, the kinase StkP controls cellular cytokinesis and morphogenesis [8,9]. In *B. subtilis*, the kinase PrkC controls spore germination [10], while the kinase YabT controls DNA proofreading and forespore metabolic quiescence during spore formation [11–13]. In *M. xanthus*, development of fruiting bodies and spores is controlled by a key transcriptional regulator MrpC, which is regulated via phosphorylation by the kinase Pkn14 [14]. Interestingly, MrpC is also controlled by a two component system MrpA/MrpB. It has recently been highlighted that bacterial Hanks-type kinases can phosphorylate transcription regulators and histidine kinases of the two component systems, and thus contribute to signal integration in these otherwise linear signal transduction pathways [15].

Regarding the evolutionary origin of bacterial Hanks-type Ser/Thr kinases, in 1998, Leonard *et al.* [16] argued that these putative bacterial kinases constitute novel families of the “eukaryotic” protein kinase superfamily. The authors proposed an evolutionary scenario supporting a monophyletic origin of this entire kinase superfamily, which appeared before the separation of the three domains of life. However, despite the evidence of a monophyletic origin, before the emergence of eukarya, the term “eukaryotic-type kinases” unfortunately propagated in the literature. We have in the past argued that the term “eukaryotic-type kinase” is a misnomer when describing Hanks-type kinases in bacteria [17,18]. Our argument was based on the fact that these kinases are present in bacteria and archaea, and that there is no evidence supporting the notion that they originated in eukarya. Nevertheless, the term “eukaryotic-type kinase” remains in use until this day, especially among microbiologists [19–24]. In order to further substantiate our claim that Hanks-type kinases are an ancient protein family, shared by archaea, bacteria and eukarya, we here attempted to provide a new line of evidence, independent from that of Leonard *et al.* [16]. For this, we decided to take a phylostratigraphic approach [25], a method for tracing the origin of evolutionary novelties based on the similarity searches of a well-populated protein sequence database. The phylostratigraphic approach has been successfully applied to trace the evolutionary origin of cancer-related genes [26], for precise evolutionary stratification of developmental phenomena [27], and in studies of evolutionary emergence of *de novo* genes from non-genic regions [28]. Theoretical background, advantages and limits of this method have been recently covered by Domazet-Lošo *et al.* [29].

In analogy to the study of Hanks *et al.* [1], where the starting point was the ensemble of characterized

kinases from eukarya, our point of departure was the set of experimentally characterized Hanks-type kinases from bacteria. We focused on the 30 well-characterized bacterial Hanks-type kinases, as listed by Pereira *et al.* [4], with the addition of two recently characterized *B. subtilis* kinases YabT [11] and PrkD [30]. Complete sequences of these 32 kinases were obtained from the National Center for Biotechnology Information (NCBI) (Supplementary Table 1) and subjected to the analytical pipeline presented in Supplementary Fig. 1. Briefly, the kinase catalytic domain sequences were aligned and used to construct a hidden Markov model consensus profile. The consensus sequence profile was used to search UniProtKB/Swiss-Prot, the manually annotated section of the UniProt databases, under a stringent cutoff (*E*-value of e^{-5}). The query returned 1457 Ser/Thr protein kinase sequences (Supplementary Table 2), from a total of 164 genomes, covering species from all three domains of life: eukarya, bacteria and archaea (Supplementary Table 3). Figure 1a presents a multiple sequence alignment of a subset of catalytic domains from all three domains of life, showing that the Hanks motifs [1] are well conserved in these kinases from archaea, bacteria and eukarya. Sequences of bacterial HipA kinases, distantly related to the Hanks-type kinases [31], were included to highlight the core signature motifs of Hanks-type kinases.

Next, to determine their phylogenetic origin, the sequences of the 32 experimentally characterized bacterial Ser/Thr kinases (Supplementary Table 1) and 1457 of their closest homologues from UniProtKB/Swiss-Prot (Supplementary Table 2) were subjected to a phylostratigraphic analysis, as described previously [26–28]. We first built a custom protein database by combining complete genomes from NCBI, Ensembl and Joint Genome Institute. In total, 113,834,351 protein sequences from 25,223 genomes were collected. We searched the protein kinase sequences against this custom protein database by using BLASTP at *E*-value cutoff of e^{-3} [26]. Using the obtained BLAST output, we determined the phylogenetic origin of the kinase genes on a premade consensus phylogenetic tree. Our phylostratigraphic analysis placed all of the 32 experimentally characterized bacterial Ser/Thr kinases unambiguously in the phylostratum 1. This is the phylogenetic level corresponding to the lineage leading to the last universal common ancestor (LUCA), that is, prior to separation of the three domains of life (Supplementary Table 1). In the consensus phylogenetic tree constructed for analyzing bacterial kinases, this phylostratum is represented by reference genomes of 256 archaeal and 1458 eukaryal species. Essentially all 32 bacterial kinases had significant hits within archaeal (97%) and eukaryal (100%) sequences, and on average, each kinase had significant hits to 32% archaeal and 92% eukaryal species in the database. A very similar

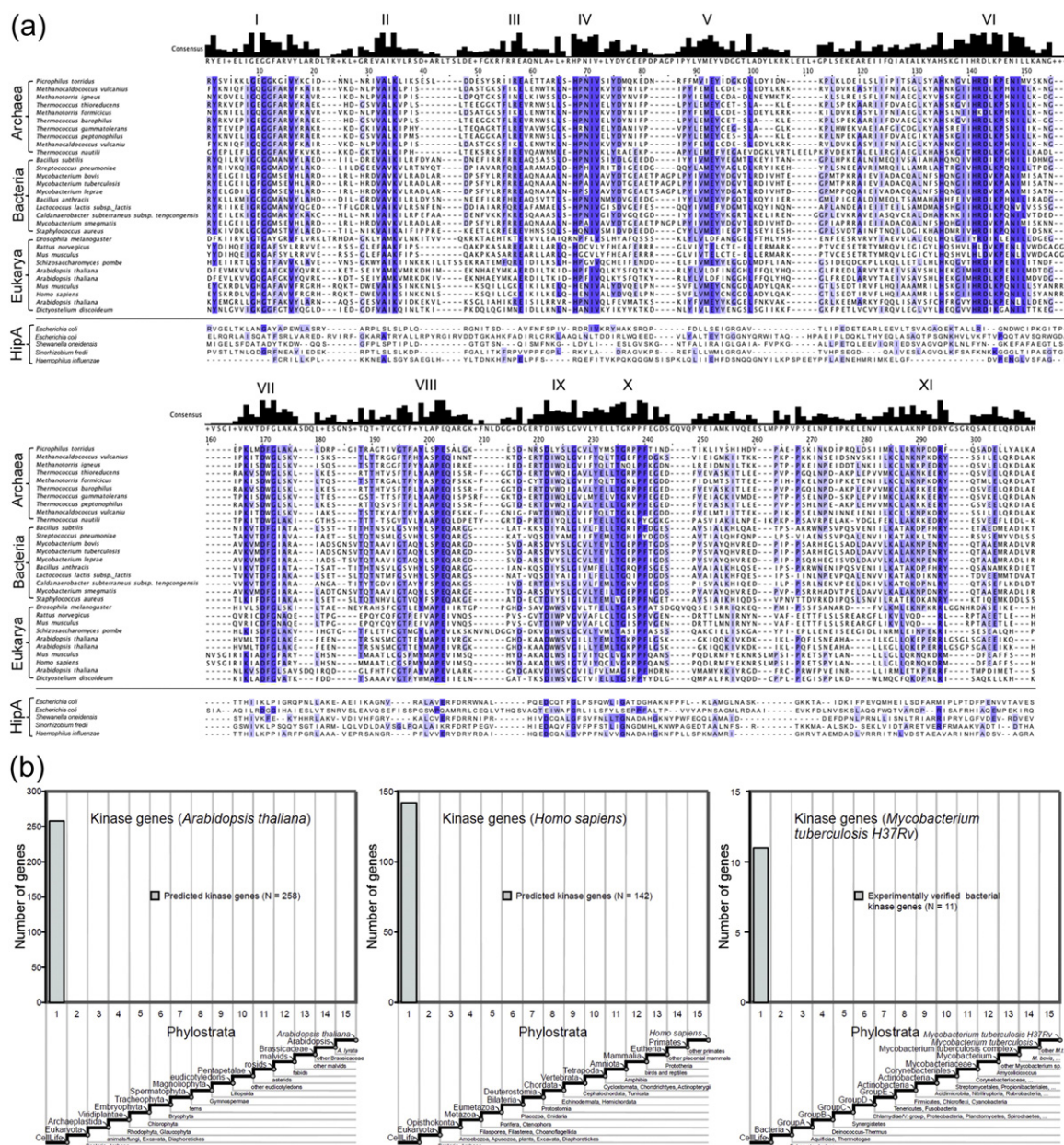


Fig. 1. Hanks-type kinase are equally conserved among the three domains of life, and most of them have originated in LUCA. (a) Multiple alignment of the catalytic domains of Hanks-type kinases from archaea, bacteria and eukarya, respectively. Sequences were randomly selected from the data set of 1457 sequences retrieved from UniProtKB/Swiss-Prot. Blue shading corresponds to highly conserved motifs, as defined by Hanks *et al.* [1], marked by roman numerals. Five sequences of HipA kinases, distantly related to Hanks-type kinases, are included at the bottom of the alignment. Kinases represented in this alignment, listed with their species of origin and UniProt codes are as follows: *Picrophilus torridus* (Q6L2T2), *Methanocaldococcus vulcanius* (C9RH09), *Methanoterris igneus* (F6BEU8), *Thermococcus thioreducens* (A0A0Q2RDP2), *Methanoterris formicicus* (H1L0Y3), *Thermococcus barophilus* (F0LHW7), *Thermococcus gammatolerans* (C5A5G9), *Thermococcus peptonophilus* (A0A142CXX3), *M. vulcanius* (C9RGZ6), *Thermococcus nautili* (W8NTA3), *Bacillus subtilis* (O34507), *Streptococcus pneumoniae* (Q8KY50), *Mycobacterium bovis* (P0A5S5), *Mycobacterium tuberculosis* (P9W181), *Mycobacterium leprae* (P54744), *Bacillus anthracis* (Q81WH6), *Lactococcus lactis* subsp. *lactis* (Q9CEF5), *Caldanaerobacter subterraneus* subsp. *tengcongensis* (Q8R9T6), *Mycobacterium smegmatis* (A0QNG1), *Staphylococcus aureus* (A6QGC0), *Drosophila melanogaster* (Q9V315), *Rattus norvegicus* (Q63638), *Mus musculus* (Q62407), *Schizosaccharomyces pombe* (Q09170), *Arabidopsis thaliana* (P42818), *A. thaliana* (Q39030), *Mus musculus* (Q9QY01), *Homo sapiens* (Q81YT8), *A. thaliana* (Q9FJ55), *Dictyostelium discoideum* (Q8T218), *Eshcherichia coli* (P23874), *E. coli* (P39410), *Shewanella oneidensis* (Q8EIX3), *Sinorhizobium fredii* (P55412) and *Haemophilus influenzae* (P44033). (b) Phylostratigraphy analysis of Hanks-type kinases from *A. thaliana*, *H. sapiens* and *M. tuberculosis*. Origin of individual sequences is mapped on the reference evolutionary tree for each species.

outcome was obtained with the 1457 kinases extracted by the UniProt search. A total of 1453 kinases from this data set (99.7%) were placed in the phylostratum 1. The remaining four kinases were found in the phylostratum 2 (the lineage leading to the origin of eukarya), and they are as follows: Bub1 from *Schizosaccharomyces pombe* and 7tmk2, Sky1 and Scy2 from *Dictyostelium discoideum*. To illustrate these results, phylostratigraphic maps are shown for three organisms harboring a large number of kinase genes: *Homo sapiens*, *Arabidopsis thaliana* and *M. tuberculosis* (Fig. 1b). All of their kinases map consistently to the phylostratum 1. As previously proposed by Leonard *et al.* [16], our analysis clearly confirmed that this protein superfamily originated in LUCA and is comparably spread in bacteria, eukarya and archaea.

It is known that some genes of eukaryal origin can end up in genomes of bacterial pathogens and commensals by horizontal gene transfer [32–34]. It is therefore possible that some Hanks-type kinase genes have been transferred, for example, from humans to their bacterial commensals. However, our examination of available extensive metagenome data sets of human gut microbiota [35] did not reveal any particular enrichment of Hanks-type kinases in human commensals. Moreover, we performed a comprehensive analysis of 967 publicly available bacterial genomes

encoding Hanks-type kinase homologues (1475 putative kinase genes), to assess whether they have any significant association to the sites of integron-mediated gene transfer [36]. Only 0.1% of the identified putative kinase genes could be associated to these gene transfer sites. Finally, we asked whether phylogenetic trees based on Hanks-type kinase sequences would differ significantly from standard phylogenetic trees based on small subunit ribosomal RNAs. Extensive gene transfer would be expected to alter the topology of the kinase-based trees. We generated corresponding phylogenetic trees from our entire kinase data set, and the topology of the kinase-based trees matched closely with the topology of rRNA-based trees. A representative tree comparison is shown in Supplementary Fig. 2. All these findings support the notion that Hanks-type kinase gene transfer most likely did not occur on a large scale.

Finally, we asked whether the present-day Hanks-type kinase superfamily could have appeared through convergent evolution. Is it possible that bacterial Hanks-type kinases arose from some ancestral bacterial kinase, and through the process of convergent evolution obtained a catalytic site similar to their counterpart protein kinases in archaea and eukarya? In order to examine that hypothesis, we compared the available tertiary structures of bacterial Hanks-type kinases to some of their bacterial and eukaryal

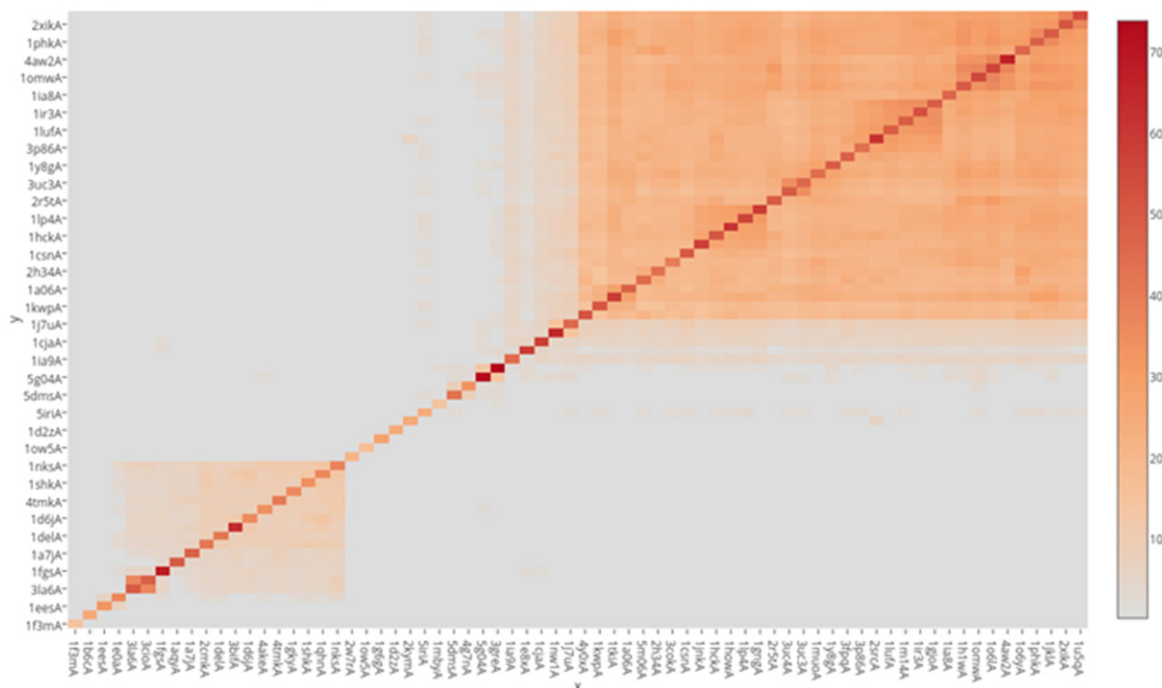


Fig. 2. Structural similarity matrix (Dali Z-scores) obtained from the Dali server [40]. The heat map represents the values of the Dali Z-score, a pairwise similarity metric. The bottom left square is constituted of 13 P-loop kinases [38] and 2 BY-kinases [39]. The larger upper right square is constituted of Hanks-type kinases of bacterial [27] and eukaryal [32] origin. The UniProt identifiers and PDB codes of the kinases used for this analysis, along with species names, are provided in Supplementary Table 4.

counterparts, aiming to reconstruct their phylogenetic relationships based on structural alignments [37]. For bacterial kinases, we used 13 available structures of P-loop kinases, enzymes that phosphorylate nucleosides, nucleotides, sugars, and coenzyme precursors [38], and 2 bacterial protein-tyrosine kinases (BY-kinases) [39]. For kinases of eukaryal origin, we selected 31 structures of eukaryal protein kinases from the study by Scheeff and Bourne [37]. These were compared to the 26 available structures of characterized bacterial Hanks-type kinases from our data set. We performed an all against all structure comparison using the Dali server [40,41]. The results, presented as a structural similarity matrix in Fig. 2, clearly indicate that bacterial Hanks-type kinases cluster with their eukaryal counterparts, and their structures are quite distinct from bacterial P-loop kinases and BY-kinases. The bacterial P-loop and BY-kinases, for which a common evolutionary origin has been suggested [39], cluster together in our analysis of tertiary structures. The results of the same comparison using the Dali server are also provided as a dendrogram and a correspondence analysis (Supplementary Fig. 3). These results clearly support a monophyletic origin of Hanks-type kinases and are not consistent with the hypothesis of convergent evolution, whereby bacterial Hanks-type kinases could have evolved from an ancestor of P-loop and BY-kinases.

Based on the presented evidence, we conclude that the Hanks-type kinases are an ancient and ubiquitous protein family with a monophyletic origin, in no way linked specifically to eukarya. Therefore, we propose that the term Hanks-type kinases should be adopted to replace the misleading term “eukaryotic-type kinases,” and all derivatives thereof.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jmb.2017.11.004>.

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†These authors contributed equally.

Abbreviations used:

LUCA, last universal common ancestor; NCBI, National Center for Biotechnology Information.

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